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Subject: Acute and subacute (chronic) inhalation toxicity
of Aerosol Ristex C.

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PROCEDURE:

A. Acute Inhalation Toxicity. Seven adult, male guinea pigs, weighing between 500 and 700 grams, were selected and numbered 2-8. Animals 2 and 3 served as controls while animals 4-8 served as experimentals and received the following treatment. The guinea pigs were immobilized in a plastic, plywood, and wire gauze chamber. The chamber was constructed so as to hold 5 animals at one time. This device allowed the release of the aerosol preparation around the head of the animal while avoiding the stream of released spray to be aimed directly into the respiratory passage. The restraining device, containing the 5 guinea pigs to be treated, was placed in a glove box. They were then subjected to a 30 second continuous spray release of aerosol Ristex C and allowed to remain in the sprayed atmosphere for 15 minutes. At 30-minute intervals the procedure was repeated until a total of 10 successive exposures had been completed.

Upon release, the animals were cleaned of extraneous spray material on their fur and observed closely for 4 days with respect to symptomatology, food intake, water intake, body weight, and hematology. In addition, radiographs were taken the day before the treatment and again the fourth day of the observation period. Blood samples were taken via heart puncture on the day before treatment and on the fourth day after treatment. These blood samples were analyzed in duplicate for: red cell count, white cell count, differential cell count, hemoglobin, and hematocrit. The red and white cell counts were obtained using a Coulter Counter. Body weights were also taken at selected intervals.

At the end of the 4 day period of observation following treatment, the animals were sacrificed and tissue samples from the trachea, lung, and liver were taken. These tissues were fixed in Bouin's solution, embedded in paraffin, and routinely stained with Harris' hematoxylin and eosin.

The two control animals received the same treatment except that they were not sprayed with the aerosol Ristex C.

B. Subacute (Chronic) Inhalation Toxicity. Eight adult, male guinea pigs, weighing 500-700 grams were selected and numbered 1 and 9-15. Animals 9 and 10 served as controls and animals 1 and 11-15 served as experimentals. The experimental animals were restrained in the same device as described above and were given two 30-second continuous spray exposures daily (usually several hours apart). The treatments were administered in the glove box described above and were performed daily for 101 days with this exception. On day 65 of treatment, it was observed that one control animal (No. 10) was dead and one experimental animal (No. 14) was very sick. The weather had turned cold a few days earlier and the temperature in the laboratory had also dropped. It was theorized that the animals had contracted an infection. For the following 4 days no treatment was given and an antibiotic was administered in the drinking water of all animals. On day 67 the experimental animal (No. 14) died. On day 69, all surviving animals appeared to have recovered and the treatment with Ristex was resumed and the antibiotic was no longer given.

During the 101 day duration of the experiment, the animals were observed closely with respect to symptomatology, food intake, water intake, and body weight. Radiographs were taken a week prior to the initiation of treatment and again at the termination of the experiment. Blood samples were also taken a week prior to the first treatment and again at the end of the experiment. These samples were taken and analyzed as described above. At the termination of this experiment, tissues were removed and prepared for histological examination as described previously.

RESULTS:

The data was collected, tabulated, averaged, and entered in graph or tabular form in the following pages. The values obtained from the blood study are given as well as a per cent change. This is either a plus or minus value and is based on the pre-treatment value obtained for each animal or group of animals. The tissues were examined and photographs taken of a representative sample of each treatment group. These photographs are included as Plates I, II, and III. The radiographs, each bearing the number of the animal, are included. The pre-treatment radiograph does not have a clipped corner whereas the post-treatment radiograph has a clipped corner. Differential white cell counts are being evaluated and are not included in this report.

A. Acute Inhalation Toxicity. Weight changes. (Fig. I) The weight changes of the control and experimental groups parallel each other except for the short interval following treatment when the experimental animals lost weight. It is felt that excessive salivation during the treatment period may have caused the temporary reduction in body weight.

Blood Studies. (Tables I and II) White cell count--all values obtained are within the limits set forth as normal (see Appendix). Red cell count--the values obtained were slightly lower than the normal values (see Appendix), but all values were consistent in both experimental and control groups alike. Both groups showed a decrease in red cell counts and was probably due to the short time interval between the first blood sampling and the second. Approximately 0.75 ml of blood was taken in each instance and the animals may have been rendered slightly anemic for a short time following the first sampling. Hematocrit--all values of hematocrit fall within the normal range (see Appendix). Hemoglobin--with one exception, the hemoglobin values fall within the normal range (see Appendix). This exception is animal number 8. In comparing the hemoglobin, hematocrit and red cell counts, it becomes apparent that this animal was anemic before the experiment began and was only slightly more anemic at the end of the experiment.

Histology (Plates I, II, and III). There were no observable differences in the histology of the lung, trachea, or liver in the experimental and control animals. At sacrifice the internal organs of both groups appeared normal.

Radiographs. There appears to be no appreciable differences in the radiographs taken before and after treatment. Minor differences in contrast of the radiographs is due to a stronger developer used on the post-treatment radiographs.

Water and food consumption. Food consumption was the same for both groups, but the water consumption was slightly greater in the experimental animals. All animals appeared normal in all physical respects.

B. Subacute (Chronic) Inhalation Toxicity. Weight changes. (Fig. II) The weights of the two groups of animals roughly parallel each other. It is noted that both groups lost weight toward the end of the experiment (days 84-101). The cause of this weight loss is undetermined, but as it occurred in both groups, it would seem that the treatment was not the cause.

Blood studies. (Tables III and IV) White cell count--all values except for animals 9 (a control) and 15 (an experimental) are within normal limits. Animal 9 originally was slightly leucopenic but was normal at the termination of the experiment. Animal 15 was leucopenic in both instances. Red cell count. Nine of the 12 samples taken were below the expected normal (see Appendix). It is interesting to note, however, that the red cells increased in both groups during the experiment. Perhaps prior conditions had left the animals slightly anemic before they were obtained for this experiment.

Hematocrit--all values are within normal range (see Appendix).
Hemoglobin--all values are within normal range (see Appendix).

Histology (Plates I, II, and III). There were no observable differences in the histology of the lung, trachea, or liver in the experimental and control animals. At sacrifice the internal organs of both groups appeared normal.

Radiographs. There appears to be no appreciable differences in the radiographs taken before and after treatment. The body size in the later radiographs is smaller--this agrees with the data concerned with body weights.

Water and food consumption. There was no appreciable difference between experimental and control groups with respect to food and water consumption. All animals in the chronic study appeared normal in all respects except as noted above.

CONCLUSIONS:

Aerosol Ristex C when used as described above did not produce any positive toxicity symptoms in the guinea pig. Based upon the data presented above, it would seem that the Aerosol Ristex C should be relatively non-toxic to other mammalian species if an allowance is made for species differences and if an allowance is made for a margin of safety.

Eddie D. Leach

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9 3
5 2
3 2
2 2

APPENDIX

Normal blood values for the guinea pig. From: HANDBOOK OF BIOLOGICAL DATA, Federation of American Societies for Experimental Biology and Medicine. Altman and Dittmer, eds. 1964.

Red Cell Count 5.6 (4.5-7.0) $\times 10^6/\text{mm}^3$

Hematocrit 37-47%

Hemoglobin 14.4 (11.0-16.5) grams/100 ml of blood

White cell count 10,000 (7,000-19,000)/ mm^3

Differential count

Neutrophils 42(22-50)%

Eosinophils 4 (2-12)%

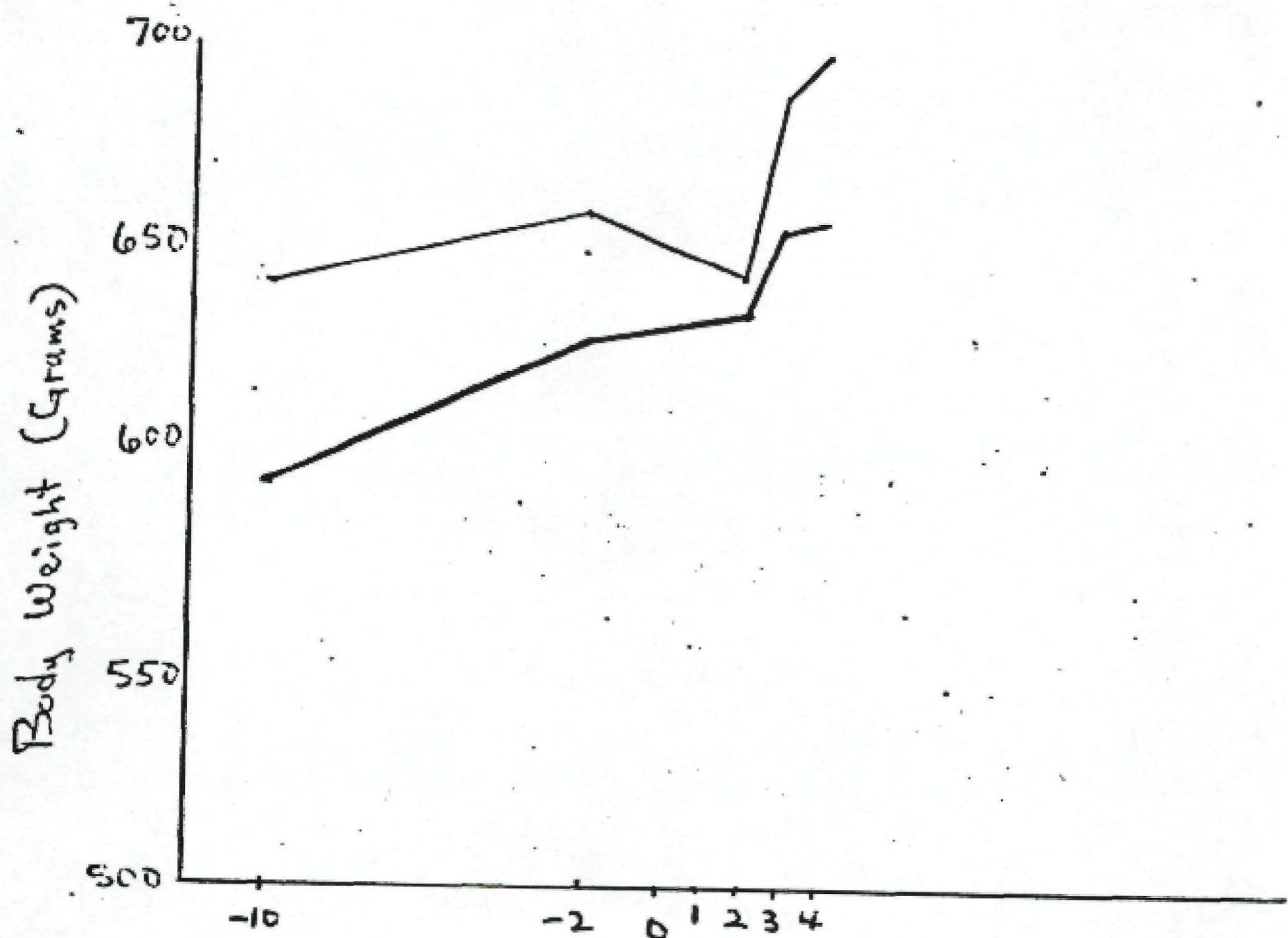
Basophils 0.7 (0-2)%

Lymphocytes 49 (37-46)%

Monocytes 4.3 (3-13)%

9522

Average
Fig. I. Weight Changes in the
Acute Inhalation Study.



23 23

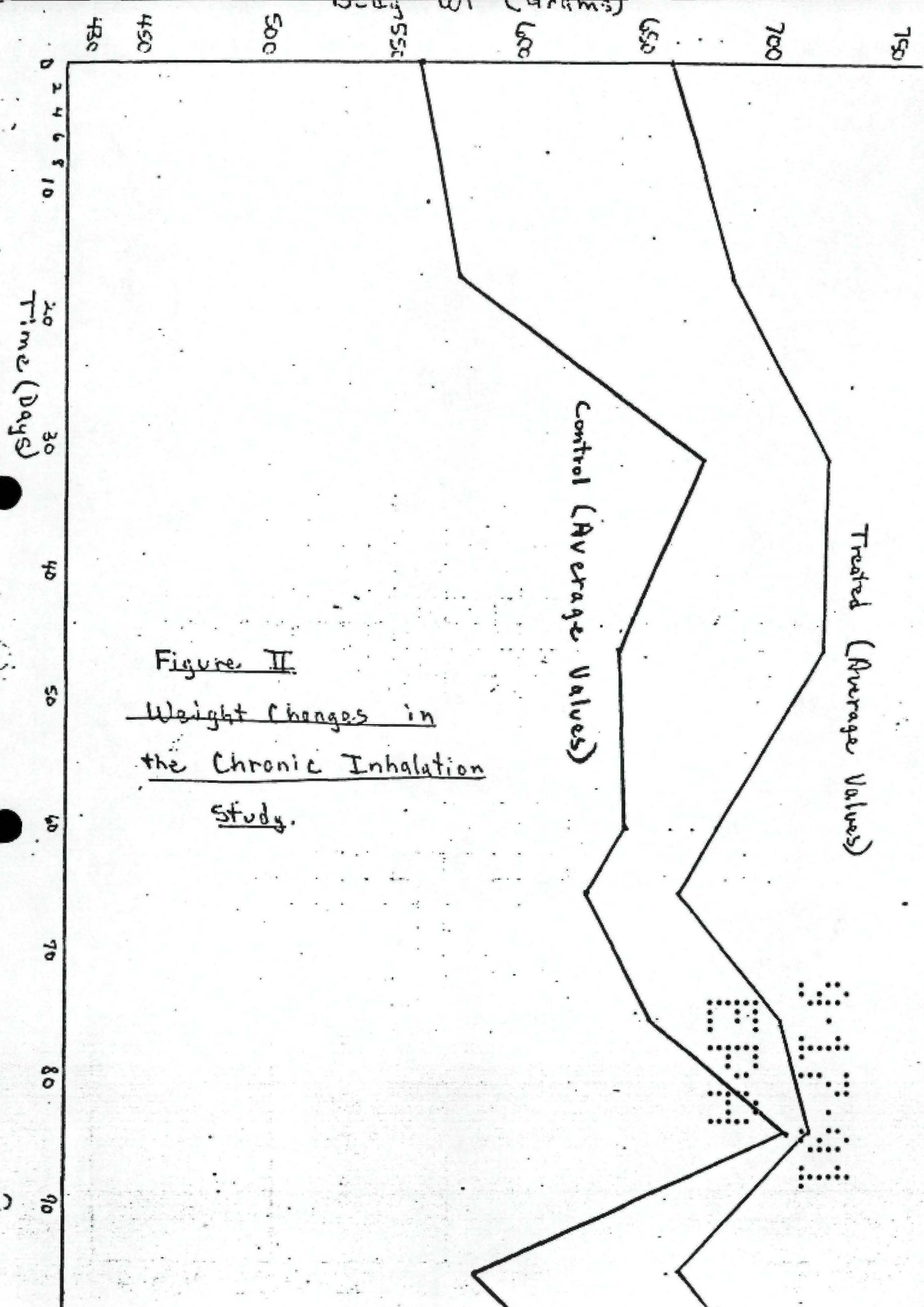


Figure II
Weight Changes in
the Chronic Inhalation
Study.

Table I

Acute Study Blood Cell Counts*

Treatment	Animal No.	White Cell Count		Avg. % Chg.
		Before	After	
Control	2	11,442	8,531	Avg. - 10.64%
	3	13,923 Avg. 12,682	14,133 Avg. 11,332	
Treated	4	11,538	12,012	Avg. + 7.24%
	5	9,187	11,432	
	6	9,790	11,716	
	7	9,556	8,273	
	8	10,445 Avg. 10,103	10,742 Avg. 10,835	
		Red Cell Count		
		Before	After	
Control	2	4.29×10^6	4.12×10^6	Avg. - 4.04%
	3	4.10×10^6 Avg. 4.20×10^6	3.94×10^6 Avg. 4.03×10^6	
Treated	4	4.42×10^6	3.73×10^6	Avg. - 4.18%
	5	4.29×10^6	4.23×10^6	
	6	3.96×10^6	4.22×10^6	
	7	4.26×10^6	4.21×10^6	
	8	3.36×10^6 Avg. 4.06×10^6	3.04×10^6 Avg. 3.89×10^6	

* Cell counts are expressed as the number of cells/mm³

Acute Study - Hematocrit

Treatment	Animal No.	Hematocrit		Percent Change
		Before	After	
Control	2	46.5	44.8	- 3.70%
	3	40.9	42.5	+ 3.90%
		Avg. 43.7	Avg. 43.7	<u>avg. +0.</u>
Treated	4	48.6	39.1	- 19.0%
	5	46.0	45.0	- 2.20%
	6	42.0	46.7	+ 11.49%
	7	45.2	43.22	- 4.38%
	8	35.9	32.08	- 10.64
		Avg. 43.5	41.2	<u>avg. -5.01</u>

Acute Study - Hemoglobin *

Treatment	Animal No.	Hemoglobin		Percent Change
		Before	After	
Control	2	13.5	12.9	- 4.4
	3	12.0 Avg. 12.75	10.9 Avg. 11.9	- 9.16 Avg. - 6.7
Treated	4	12.7	11.8	- 7.05
	5	12.9	12.7	- 1.55
	6	11.6	12.9	+ 11.2
	7	12.0	12.1	+ 0.83
	8	9.6	9.5	- 1.04
		Avg. 11.76	Avg. 11.8	<u>Avg. +.47</u>

* Grams of Hb/100 ml Blood.

Table III.

Chronic Study

Blood Cell Counts *

Treatment	Animal No.	White Cell Count		Per Cent Change
		Before	After	
Control	9	6,771	8,029	+18.57%
Treated	1	9,680	7,377	
	13	7,355	6,085	
	11	12,373	9,320	
	12	13,240	16,819	
	15	4,860	4,994	
		Avg. 9,502	Avg. 8,919	Avg. -6.13%
		Red Cell Count		
		Before	After	
Control	9	4.07×10^6	4.22×10^6	+3.68%
Treated	1	4.74×10^6	4.37×10^6	
	13	3.84×10^6	4.28×10^6	
	11	3.75×10^6	4.84×10^6	
	12	3.29×10^6	4.38×10^6	
	15	3.43×10^6	5.38×10^6	
		Avg. 3.81×10^6	Avg. 4.45×10^6	+22.04%

* Cell counts are the number of cells/mm³ of whole blood.

Table IV

Chronic Study — Hematocrit

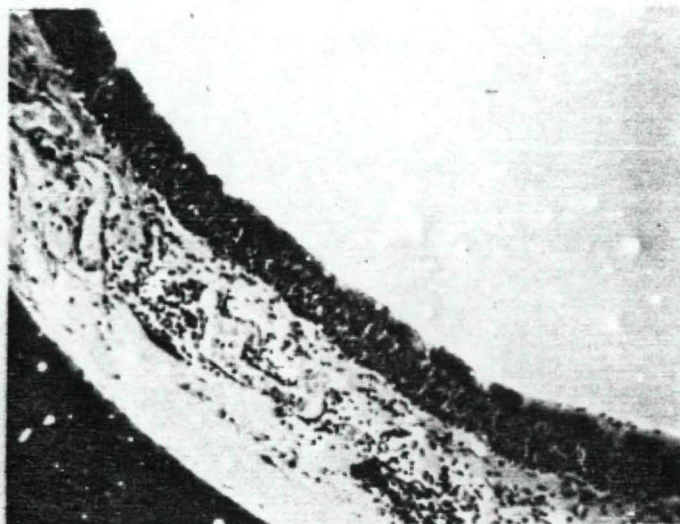
Treatment	Animal No.	Hematocrit		Per Cent Change
		Before	After	
Control	9	45.0	44.2	- 1.77 %
Treated	1	44.9	46.1	
	13	47.5	47.0	
	11	44.0	43.1	
	12	40.5	43.2	
	15	40.5	56.3	
		<u>Avg. 43.5</u>	<u>Avg. 47.1</u>	Avg. change + 8.27 %

Chronic Study — Hemoglobin*

Treatment	Animal No.	Hemoglobin		Percent Change
		Before	After	
Control	9	14.1	11.6	- 17.73 %
Treated	1	13.4	12.5	
	13	14.1	12.9	
	11	13.7	13.1	
	12	12.2	12.5	
	15	12.2	15.2	
		<u>Avg. 13.12</u>	<u>Avg. 13.24</u>	Avg. change + 0.91 %

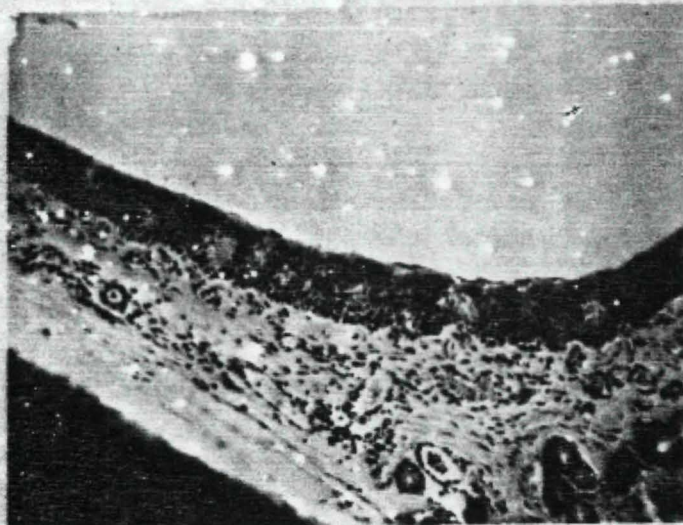
* Grams of Hb/100 ml Blood

Plate I



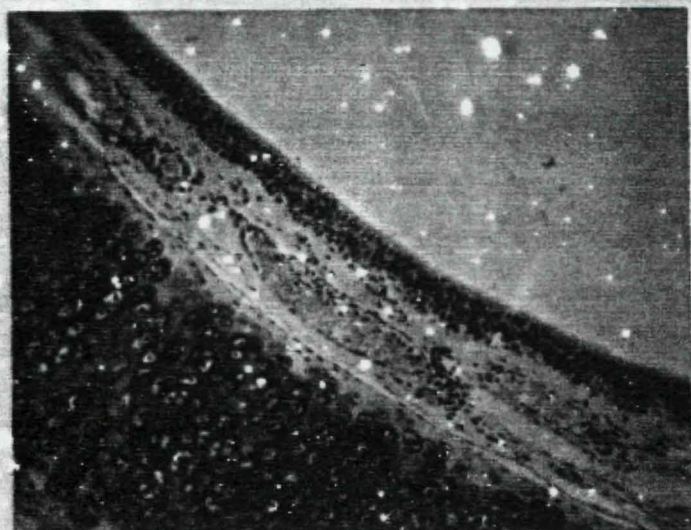
Control
Trachea

100X.



Trachea from
animal #5 of the
acute study.

100X.



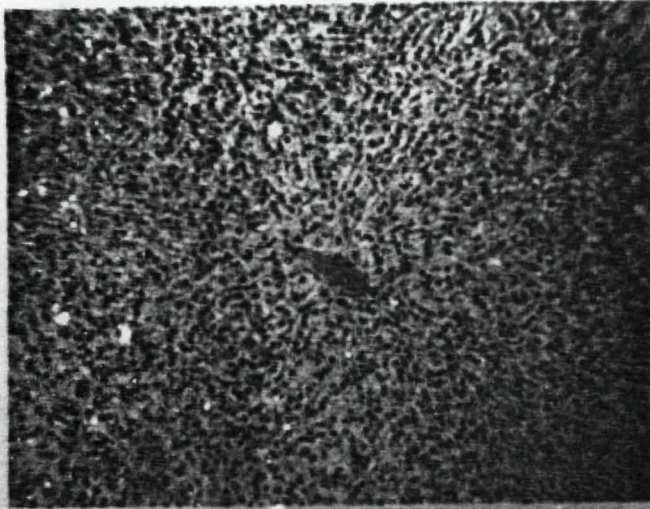
Trachea... from
animal #15 of
the chronic study.

100X.



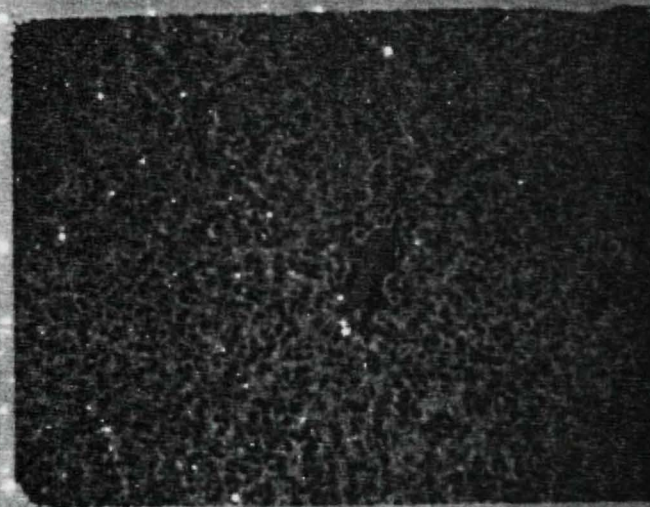
MILLIGAN COLLEGE

Plate II



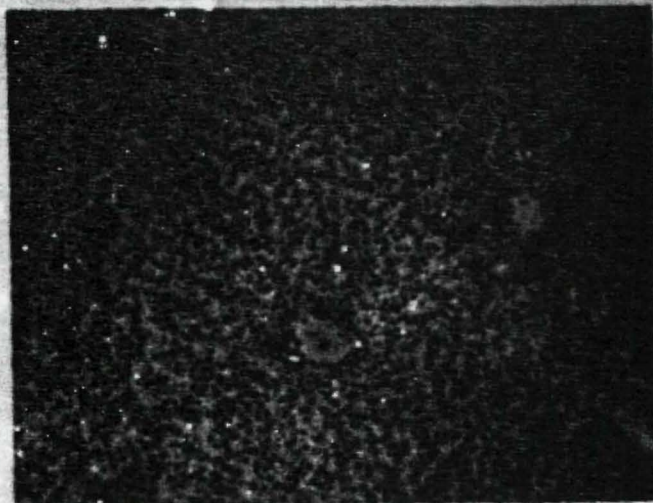
Control
Liver

100x.



Liver from
animal # 5 of the
acute study.

100x.



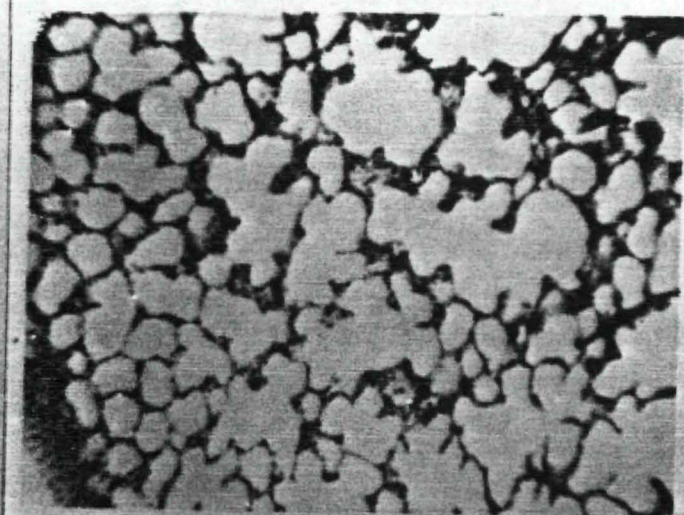
Liver...from
animal...# 12...of the
chronic...study.

100x.

Plate III

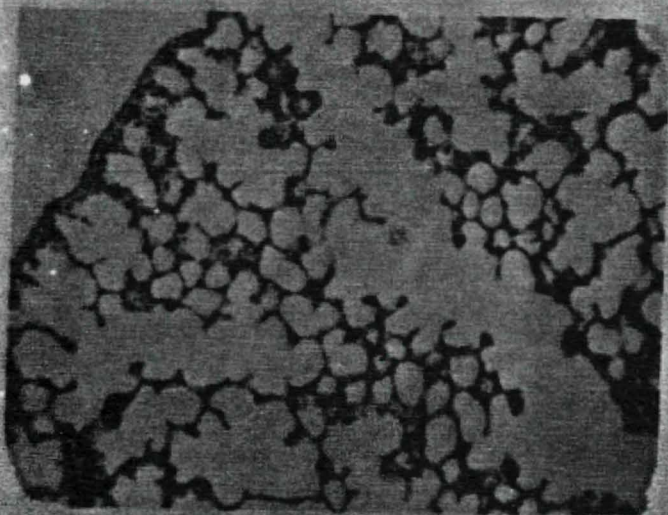
Control lung

100X.



Lung from
animal # 5
of the acute
study.

100X.



Lung from
animal # 15
of the chronic
study.

100X.

